Reye syndrome—insights on causation and prognosis

Reye syndrome (RS) is an abrupt insult to mitochondria manifesting as acute encephalopathy, selective hepatic dysfunction, and fatty infiltration of the viscera—as originally described in 1963 (see Wood). Causation, however, remains unclear, especially the role of aspirin in possible pathogenesis. Although prompt recognition and intensive therapy is essential to full recovery, a paucity of cases in recent years has meant that few younger paediatricians have any personal experience. This and a spate of recent literature—clinical and scientific—has prompted this review.

Background
RS is a biphasic illness. A viral prodrome—with infection of the upper respiratory tract or bowel, or varicella—is followed several days later by an abrupt onset of encephalopathy heralded as profuse, effortless vomiting. The rate and degree of neurological decline vary. Raised intracranial pressure from brain swelling is usually thought to cause death or neurological injury.

The British Paediatric Surveillance Unit defines RS as an unexplained, non-inflammatory encephalopathy in those less than 16 year of age, associated with a serum ammonia and free fatty acids, and develop hepatic dysfunction, and fatty infiltration and mitochondrial damage. Immature macrophages of tumour necrosis factor (TNF), a known mediator of metabolic toxicity. In rat hepatocytes, for example, TNF inhibits fatty acid oxidation; influenza B virus produces similar effects in mice. Following sublethal doses of endotoxin, rats have increased concentrations of plasma ammonia and free fatty acids, and develop hepatic fatty infiltration and mitochondrial damage. Immature rodents appear more sensitive to the effects of TNF. Aspirin enhances in vitro release of TNF by mouse macrophages. Finally, aspirin and salicylate at 1–5 mM directly inhibit release of the proinflammatory and antiapoptotic nuclear factor kB, allowing target cells to proceed more rapidly down the pathway to cell death. Thus viral infection may influence cellular and subcellular events via immune mediators.

Mitochondrial failure
The clinical findings in RS are compatible with hepatotoxicity caused by mitochondrial failure. Mitochondria provide most of the energy for liver cell function via oxidative phosphorylation fed by the tricarboxylic acid cycle and

Aspirin metabolism
Following absorption, aspirin is metabolised rapidly to salicylate. At usual anti-inflammatory doses, with plasma concentrations of 1–5 mM, the half life approximates to 12 hours; at high therapeutic doses it may reach 30 hours. Salicylate is metabolised within hepatocyte mitochondria and endoplasmic reticulum to significant amounts of hydroxysalicylate (HSA) and gentisate. Among 56 patients with RS in Northern Ireland (1979–86), the serum salicylate range and mean on admission were 0–2.5 and 0.9 mM/L respectively (JG, unpublished data). This concurs with published data; HSA concentrations tend to be twofold higher. In RS, therefore, aspirin metabolites may persist for some time at millimolar concentrations. Are such concentrations capable of influencing events, however?

Viral infection and the immune response
Antecedent viral infection appears to be a sine qua non for development of RS. Viral RNA frequently diverts host endoplasmic reticulum to biosynthesis of viral protein. A number of virus infections disturb Kupffer cell function and endotoxaemia commonly results, triggering a cascade of cytokines. Endotoxin like activity has been reported in plasma and CSF in RS. Endotoxin provokes release by macrophages of tumour necrosis factor (TNF), a known mediator of metabolic toxicity. In rat hepatocytes, for example, TNF inhibits fatty acid oxidation; influenza B virus produces similar effects in mice. Following sublethal doses of endotoxin, rats have increased concentrations of plasma ammonia and free fatty acids, and develop hepatic fatty infiltration and mitochondrial damage. Immature rodents appear more sensitive to the effects of TNF. Aspirin enhances in vitro release of TNF by mouse macrophages. Finally, aspirin and salicylate at 1–5 mM directly inhibit release of the proinflammatory and antiapoptotic nuclear factor kB, allowing target cells to proceed more rapidly down the pathway to cell death. Thus viral infection may influence cellular and subcellular events via immune mediators.

Aspirin—a co-factor?
The evidence on prodromal aspirin and RS has been reviewed. Several case–control studies assert that the association is strong, consistent, and unbiased; this is especially true of the very rigorous “Yale” study, in which a dose–response effect was also noted. Even a total dose less than 45 mg/kg increased the risk of RS by 20-fold—the authors concluding that “no safe dose [of aspirin] exists”. No other exogenous agent has been implicated in similar well designed studies (see Hall).

Longitudinal surveillance provides further evidence. A recent review of 1207 cases reported to the National RS Surveillance System (NRSSS) at the Centers for Disease Control and Prevention, Atlanta, USA (1 December 1980 to 30 November 1997) identified 555 cases in 1980, but only two annually between 1994 and 1997; case reporting was to the 18th birthday. In the UK, the annual incidence declined from 81 cases in 1983–84 to five in 1996–97. One elegant study showed that this decline mirrored the upsurge in publications mentioning the link with aspirin that began in the USA in 1980. Furthermore, in Northern Ireland, febrile children admitted to hospital in 1988–89 showed a 17-fold reduction in preadmission aspirin use compared to those in 1985–86 (the Committee on Safety of Medicines warned of the aspirin risk in mid-1986). Finally, more than 400 UK cases reported between 1982 and 1990 were reviewed “blindly” using a Reye score (devised by Dr Susan Hall to quantitate features characteristic of “classical” RS) employed here in modified form. Highest scores were achieved by a distinct aspirin related subgroup that diminished significantly in number (79%) after 1986. The evidence of an association therefore seems epidemiologically sound, and the disappearance of RS consequent on the aspirin warnings was described two years ago in an editorial in the New England Journal of Medicine as “a public health triumph”. The evidence on prodromal aspirin and RS has been reviewed. Several case–control studies assert that the association is strong, consistent, and unbiased; this is especially true of the very rigorous “Yale” study, in which a dose–response effect was also noted. Even a total dose less than 45 mg/kg increased the risk of RS by 20-fold—the authors concluding that “no safe dose [of aspirin] exists”. No other exogenous agent has been implicated in similar well designed studies (see Hall).

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β oxidation of long chain fatty acids (LCFA). Mitochondria are also closely involved in cell death processes such as the mitochondrial permeability transition (MPT). This is the opening of a cyclosporin sensitive pore in the inner membrane of mitochondria leading to swelling, depolarisation, failure of oxidative phosphorylation, and cell death by apoptosis or necrosis. Recently, Lemasters’ group has shown that 0.3 mM salicylate can initiate MPT in isolated liver mitochondria, and, in cultured rat hepatocytes, 0.3–5 mM salicylate enhanced cell killing.

In RS the burden of evidence suggests that mitochondrial failure is the result of inhibition of oxidative phosphorylation and fatty acid β oxidation of LCFA. Microvesicular steatosis is a likely consequence and accumulation of tissue LCFA has recently been shown to initiate apoptosis via increased ceramide in a variety of cell types. Another factor is that toxic acyl-CoA esters initiate apoptosis via increased ceramide in a variety of cell types. The presence of generalised mitochondrial dysfunction. Thus inhibition of mitochondrial β oxidation in RS could lead to severe liver dysfunction and fatty infiltration.

Salicylate blocks β oxidation of long chain fatty acids

In the first study to use human cells, aspirin metabolites (though not aspirin itself) have been shown recently to inhibit mitochondrial β oxidation of palmitate. This was carried out in skin fibroblasts from children who had recovered from RS (also given aspirin for prodromal symptoms), and from controls. In cells from the latter, two of the principal metabolites—hydroxyhippurate (HHA) and gentisate—were more inhibitory than salicylate at concentrations below 5 mM, but RS cells were equally sensitive to all aspirin metabolites. Thus salicylate below 5 mM was a more effective inhibitor of β oxidation in RS cells than in controls.

Where is the site of inhibition?

Structural similarities between aspirin metabolites and substrates for the mitochondrial trifunctional enzyme (MTE) of β oxidation prompted us to examine whether the long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) component of MTE was the target for salicylate inhibition. Using fibroblasts from individuals with LCHAD deficiency, it was possible to show complete absence of inhibition of β oxidation by salicylate or HHA compared to control cells or RS cells, thus indicating that the LCHAD enzyme is the target for such inhibition.

A biological difference between control and Reye syndrome cells?

Our studies have also shown that fibroblasts from RS patients were significantly more sensitive to inhibition of β oxidation by salicylate than are control cells. The differences were demonstrable at concentrations well within the therapeutic range of plasma salicylate (see above). In control cells, 1 mM salicylate significantly stimulated palmitate oxidation, an effect quite opposite to that noted in RS cells, higher concentrations caused increasing inhibition in each group of cells. Stimulatory then inhibitory effects of salicylate on β oxidation were reported at similar concentrations in rat liver slices. Thus stimulatory and inhibitory effects of salicylate were independent; only the latter was due to blocking of LCHAD activity. How might this stimulation of β oxidation, at therapeutic salicylate concentrations, be induced? Salicylate, though not HHA or gentisate, is known to uncouple mitochondrial β oxidation from phosphorylation. Thus the stimulatory effects in control cells might be due to the presence of an uncoupling protein. In RS cells, it is possible that salicylate does not uncouple oxidative phosphorylation because a specific target protein is absent. This target protein we suggest is probably one of the family of mitochondrial uncouplers (UCPs) whose role is still unclear. Their expression is increased under conditions in which LCFA accumulate, prompting the suggestion that, by stimulating mitochondrial respiration they enhance β oxidation, thus protecting against apoptosis (see above). Lack of a specific UCP, however caused, might explain the difference in β oxidation response to low concentrations of salicylate between controls and LCHAD deficient cells on the one hand, and RS cells on the other—a difference that warrants further investigation. This sensitivity of RS cells, if widely expressed in body tissues, could explain the reaction of certain individuals to the action of aspirin on the MTE-LCHAD enzyme system that might contribute to the pathogenesis of RS.

Prognosis

If the neurological phase of RS resolves, visceral function is expected to recover completely within a few days. However, the severity and duration of both metabolic and hydrostatic effects on the nervous system will determine prognosis. Although pathogenesis of the brain swelling is unclear, its results can be all too evident, with elevation of intracranial pressure, and, if more severe, reduction in cerebral perfusion pressure; a cerebral perfusion pressure of 40 mm Hg or less in RS carries a very poor outcome. In the NRSSS, even small biochemical abnormalities appeared to influence mortality. Logistic regression analysis showed that, surprisingly, a blood ammonia little greater than the normal upper limit for older children was significantly associated with a risk of demise (p < 0.01)—the relative risk of death being 3.4 and of neurological complications 4.1.

In our series, more than 70% of the children achieved a good initial outcome (62% in NRSSS). This optimism must be interpreted with caution because in the UK, RS has tended to occur at a very young age (median age 14 months; median age in NRSSS, 6 years). Damage to the developing brain that results in neuropsychological deficits may only become apparent much later when maturation deviates from normal, and abilities may be simply less well acquired. Moreover mild impairments, perhaps clinically undetectable in themselves, may exert a cumulative influence on the development of other functions, leading to more significant disabilities. Hence the need for outcome studies during the school years and beyond. Among 56 patients seen between 1979 and 1986 (median age 0.8 years). Meekin and colleagues studied cognitive, emotional, and behavioural sequelae in 18 children who had recovered well enough to attend normal schools. The mean age was then 11.75 years, with each child being compared to the sibling nearest in age. On the basis of formal testing, general intellectual functioning, and verbal, and visuospatial skills were significantly reduced if RS occurred during infancy. A similar profile was evident on the measure of self esteem. Differences for measures of behavioural problems failed to reach significance. Another significant variable was the development of unconsciousness during encephalopathy. Although this was not so closely associated with less good outcomes, it was linked to relative deficits on the scale of cognitive ability; however, only those differences on the Wechsler scale of verbal abilities reached statistical significance.
Conclusion

Long term follow up in RS may in its way be as important as early recognition and optimum management, as are diagnostic investigations aimed at uncovering an IMD. Inhibition of the MTE-LCHAD enzyme system by salicylate and metabolites, and the differences shown for the first time in human material between control and RS cells, lends biological plausibility to the substantial body of epidemiological evidence linking RS with aspirin use during childhood viral illness. Our view is that current warnings about aspirin use must be maintained—with continued case monitoring—to ensure that this potentially devastating encephalopathy does not reappear and that “the public health triumph” will also be fully realised in the UK.

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